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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**
**BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

Application of: Burchard

Serial No.: 09/616,849

Filed: July 14, 2000

For: METHOD FOR DETERMINING THE SPECIFICITY AND SENSITIVITY OF OLIGONUCLEOTIDES FOR HYBRIDIZATION

Confirmation No.: 6450

Art Unit: 1634

Examiner: Forman, B. J.

Attorney Docket No: 9301-044

**BRIEF ON APPEAL FEE TRANSMITTAL**

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

An original and two copies of the applicant's Brief on Appeal in the above-entitled application are submitted herewith. The item(s) checked below apply:

- ☒ The Brief filing fee is $500.00.
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The brief filing fee is:

- ☒ Required.
- ☐ Not required. (Fee paid in prior appeal.)

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Respectfully submitted,

Date: November 7, 2006

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Enclosure

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# BRIEF ON APPEAL



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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**
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## BRIEF ON APPEAL UNDER 35 U.S.C. § 134 AND 37 C.F.R. §§ 41. 35 AND 41.37

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is an appeal under 35 U.S.C. § 134 and 37 C.F.R. §§ 41.35 and 41.37 from a final rejection mailed January 13, 2006 of claims 27, 29, 30, 33-40, 42-54, 59-67, 73-75, 84, 85 and 90-104 of the above-identified application. The Notice of Appeal was filed on April 7, 2006. Appellant submits this appeal brief accompanied by (1) a Petition for Extension of Time, accompanied by the appropriate fee; and (2) a Brief on Appeal Fee Transmittal Sheet.

## I. REAL PARTY IN INTEREST

Rosetta Inpharmatics LLC is the assignee of this application, and the real party in interest. An assignment transferring the right, title, and interest of inventor Julja Burchard to Rosetta Inpharmatics, Inc. was recorded in the U.S. Patent and Trademark Office on July 14, 2000 at Reel 010993, Frame 0334. An assignment transferring the right, title, and

interest of Rosetta Inpharmatics, Inc. to Rosetta Inpharmatics LLC was recorded in the U.S. Patent and Trademark Office on November 20, 2003 at Reel 014719, Frame 0805.

## II. RELATED APPEALS AND INTERFERENCES

There are no other appeals, interferences, or judicial proceedings known to Appellant, Appellant's legal representative, or assignee, which may be related to, directly affect or be directly affected by, or have a bearing on the Board's decision in the pending appeal.

## III. STATUS OF CLAIMS

Claims 27, 29, 30, 33-40, 42-54, 59-67, 73-75, 84, 85 and 90-104 are rejected.

Claims 1-26, 28, 31, 32, 41, 55-58, 68-72, 76-83 and 86-89 have been canceled.

Claims 27, 29, 30, 33-40, 42-54, 59-67, 73-75, 84, 85 and 90-104 are appealed.

## IV. STATUS OF AMENDMENTS

All amendments have been entered. Appellant did not file any claim amendments subsequent to the final rejection dated January 13, 2006.

## V. SUMMARY OF CLAIMED SUBJECT MATTER

The presently claimed invention as defined in independent claim 27 relates to a method for evaluating a binding property of a polynucleotide probe comprising a predetermined nucleotide base sequence that is complementary to at least a hybridizable portion of a target nucleotide sequence (see the instant specification at page 27, lines 2-11; page 19, lines 17-21; page 22, line 9 through page 23, line 14; page 24, line 20 through page

25, line 22). The method comprises determining a ratio of the amount of hybridization of polynucleotides in a first sample to the polynucleotide probe and the amount of hybridization of polynucleotides in a second sample to the polynucleotide probe (see, e.g., the instant specification at page 40, line 30, through page 41, line 36; and page 48, line 28, through page 49, line 11). The ratio is used as a measure of the binding property (see, e.g., the instant specification at page 40, line 30, through page 41, line 36). The first sample is a "specific" hybridization sample in which at least 75% of the polynucleotide molecules are polynucleotide molecules comprising said target nucleotide sequence, whereas the second sample is a "non-specific" hybridization sample which comprises a plurality of different polynucleotide molecules, each of which comprises a sequence that is different from the nucleotide sequences of any other polynucleotide molecules in the plurality of different polynucleotide molecules (see, e.g., the instant specification at page 4, lines 10-30; page 6, line 13, through page 7, line 17, page 7, lines 27-33, and page 29, lines 8-14).

The presently claimed invention as defined in independent claim 67 relates to a method for evaluating a binding property of a plurality of polynucleotide probes, each comprising a different predetermined nucleotide base sequence that is complementary to at least a hybridizable portion of the target nucleotide sequence (see, e.g., the instant specification at page 5, lines 9-21; page 27, lines 2-11; page 19, lines 17-21; page 22, line 9 through page 23, line 14; page 24, line 20 through page 25, line 22). The method comprises determining a ratio of the amount of hybridization of polynucleotides in a first sample to each of the plurality of polynucleotide probes and the amount of hybridization of polynucleotides in a second sample to each of the polynucleotide probes (see, e.g., the instant specification at page 5, lines 9-21; page 40, line 30, through page 41, line 36; and page 48, line 28, through page 49, line 11). The ratio is used as a measure of the binding

property (see, e.g., the instant specification at page 40, line 30, through page 41, line 36). The first sample is a "specific" hybridization sample in which at least 75% of the polynucleotide molecules are polynucleotide molecules comprising said target nucleotide sequence, whereas the second sample is a "non-specific" hybridization sample which comprises a plurality of different polynucleotide molecules, each of which comprises a sequence that is different from the nucleotide sequences of any other polynucleotide molecules in the plurality of different polynucleotide molecules (see, e.g., the instant specification at page 4, lines 10-30; page 6, line 13, through page 7, line 17, page 7, lines 27-33, and page 29, lines 8-14).

The presently claimed invention as defined in independent claim 91 relates to a method for evaluating a binding property of a plurality of polynucleotide probes to a target nucleotide sequence, wherein each polynucleotide probe in the plurality of polynucleotide probes comprises a different predetermined nucleotide base sequence that is complementary to at least a hybridizable portion of said target nucleotide sequence (see, e.g., the instant specification at page 5, lines 9-21; page 27, lines 2-11; page 19, lines 17-21; page 22, line 9 through page 23, line 14; page 24, line 20 through page 25, line 22). The method comprises determining a ratio of the amount of hybridization of polynucleotides in a first sample to each of the plurality of polynucleotide probes and the amount of hybridization of polynucleotides in a second sample to each of the polynucleotide probes (see, e.g., the instant specification at page 5, lines 9-21; page 40, line 30, through page 41, line 36; and page 48, line 28, through page 49, line 11). The ratio is used as a measure of the binding property (see page 40, line 30, through page 41, line 36). The first sample is a sample that comprises a plurality of polynucleotide molecules comprising the target nucleotide sequence and a plurality of polynucleotide molecules that do not comprise the target

nucleotide sequence (see, e.g., the instant specification at page 32, line 30 through page 33, line 6). The second sample is a sample that comprises a plurality of different polynucleotide molecules of different sequences and that does not comprise the target sequence, i.e., each different polynucleotide molecule in the second sample does not comprise the target nucleotide sequence (see, e.g., the instant specification at page 6, lines 23-30, page 7, lines 11-17, and page 33, lines 7-18). Thus, for example, the second sample can be a polynucleotide sample from a deletion mutant of the cell or organism, where the deletion mutant of the cell or organism does not express the target gene or gene transcript (see, e.g., the instant specification at page 7, lines 2-5).

The presently claimed invention as defined in independent claim 93 relates to a method for evaluating a binding property of a plurality of polynucleotide probes to a target nucleotide sequence, wherein each polynucleotide probe in the plurality of polynucleotide probes comprises a different predetermined nucleotide base sequence that is complementary to at least a hybridizable portion of said target nucleotide sequence (see, e.g., the instant specification at page 5, lines 9-21; page 27, lines 2-11; page 19, lines 17-21; page 22, line 9 through page 23, line 14; page 24, line 20 through page 25, line 22). The method comprises determining a ratio of the amount of hybridization of polynucleotides in a first sample to each of the plurality of polynucleotide probes and the amount of hybridization of polynucleotides in a second sample to each of the polynucleotide probes (see, e.g., the instant specification at page 40, line 30, through page 41, line 36; and page 48, line 28, through page 49, line 11). The ratio is used as a measure of the binding property (see page 40, line 30, through page 41, line 36). The first sample is a sample that comprises a plurality of polynucleotide molecules comprising the target nucleotide sequence and a plurality of polynucleotide molecules that do not comprise the target nucleotide sequence

(see, e.g., the instant specification at page 32, line 30 through page 33, line 6). The second sample is a sample that comprises a plurality of different polynucleotide molecules wherein each different polynucleotide molecule comprises a sequence that is different from the nucleotide sequence of any other polynucleotide molecule in said plurality of different polynucleotide molecules (see, e.g., the instant specification at page 33, lines 7-9 and page 30, lines 1-3).

## VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The following grounds of rejection are presented for review in this appeal:

*Firstly*, whether claims 27, 29-30, 33-40, 42-54, 59-60, 64-65, 67, 73 and 90-104 are obvious under 35 U.S.C. § 103(a) over U.S. Patent No. 4,900,659 (hereinafter "Lo") in view of Lockhart et al., 1996, Nature Biotechnology 14:1675-1680 (hereinafter "the Lockhart Article").

*Secondly*, whether claims 61-63, 66, 74-75 and 84-85 are obvious under 35 U.S.C § 103(a) over Lo in view of the Lockhart Article as applied to claims 27 and 67 and further in view of Lockhart et al., U.S. Patent No. 6,344,316 B1 (hereinafter "the Lockhart Patent").

## VII. ARGUMENT

### A. The Applicable Case Law

A finding of obviousness under 35 U.S.C. §103 requires a determination of: (1) the scope and content of the prior art; (2) the level of ordinary skill in the art; (3) the difference between the claimed subject matter and the prior art; and (4) whether the differences are such that the subject matter as a whole would have been obvious to one of ordinary skill in

the art at the time the invention was made. *Graham v. Deere* 383 U.S. 1 (1966).

The relevant inquiry is: (1) whether the prior art suggests the invention; and (2) whether the prior art provides one of ordinary skill in the art with a reasonable expectation of success. *In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988). Both the suggestion and the reasonable expectation of success must be found in the prior art. *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991).

When selective combination of prior art references is required to render obvious a subsequent invention, "[i]t is insufficient that the prior art disclosed the components of the patented device, either separately or used in other combinations; there must be some teaching, suggestion, or incentive to make the combination made by the inventor." *Northern Telecom, Inc. v. Datapoint Corp.*, 908 F2d. 931, 934 (Fed. Cir. 1990) "[T]here must be some reason for the combination other than the hindsight gleaned from the invention itself. There must be 'something in the prior art as a whole to suggest the desirability, and thus the obviousness, of making the combination.'" *Interconnect Planning Corp. v. Feil*, 774 F.2d 1132, 1143 (Fed. Cir. 1985).

The case law has been especially vigorous on guarding against using "hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." See, e.g., *In re Fine*, 837 F.2d 1071, 1074 (Fed. Cir. 1988). The Federal Circuit said in *In re Dembiczak*

> Our case law makes clear that the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is *rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references*. ... Combining prior art references without evidence of such a suggestion, teaching, or motivation simply takes the inventor's disclosure as a blueprint for piecing together the prior art to defeat patentability--the essence of hindsight.

*In re Dembiczak*, 175 F.3d 994, 999 (Fed. Cir. 1999) (emphasis added). With respect to what might meet the requirement of a showing of motivation, the Federal Circuit said that

> To prevent the use of hindsight based on the invention to defeat patentability of the invention, this court requires the examiner to show a motivation to combine the references that create the case of obviousness. In other words, *the examiner must show reasons that the skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed.*

*In re Rouffet*, 149 F.3d 1350, 1357 (Fed. Cir. 1998) (emphasis added). The Examiner must "explain what specific understanding or technological principle within the knowledge of one of ordinary skill in the art would have suggested the combination." *In re Rouffet*, 149 F.3d 1350, 1357 (Fed. Cir. 1998) (emphasis added). With respect to the sources where motivation to combine may be found, the Federal Circuit stated that "[t]his court has identified three possible sources for a motivation to combine references: the nature of the problem to be solved, the teachings of the prior art, and the knowledge of persons of ordinary skill in the art." *In re Rouffet*, 149 F.3d 1350, 1357 (Fed. Cir. 1998)

The case law further held that each reference must be evaluated as a whole, i.e., disclosures in the reference that diverge from and teach away from the invention cannot be disregarded. "Not only must the claimed invention as a whole be evaluated, but so also must the references as a whole, so that their teachings are applied in the context of their significance to a technician at the time--a technician without our knowledge of the solution." *Interconnect Planning Corp. v. Feil*, 774 F.2d 1132, 1143 (Fed. Cir. 1985). "It is *impermissible* within the framework of a Section 103 rejection *to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary to the full appreciation of what the reference fairly suggests* to one of

ordinary skill in the art." *In re Wesslau*, 353 F.2d 238, 241 (C.C.P.A. 1965) (emphasis added).

**B. The Rejection of Claims 27, 29-30, 33-40, 42-54, 59-60, 64-65, 67, 73 and 90-104 over Lo In View of the Lockhart Article Is Erroneous and Should Be Reversed**

With respect to the first issue on appeal, claims 27, 29-30, 33-40, 42-54, 59-67, 73-75, 84-85 and 90-104 are rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 4,900,659 ("Lo") in view of Lockhart et al., Nature Biotechnology 14:1675-1680, hereinafter "the Lockhart Article"). In the Office Action mailed January 13, 2006 (hereinafter the "Office Action"), the Examiner acknowledges that Lo does not teach evaluating probes having a predetermined base sequence. However, the Examiner contends that the Lockhart Article teaches a method for evaluating a polynucleotide probe having a predetermined base sequence. The Examiner contends that it would have been obvious to a person skilled in the art to apply known sequence analysis for probe selection as taught by the Lockhart Article to the probe selection method of Lo for the expected benefit of obtaining useful probes based on sequence information. Appellant respectfully submits that Lo in combination with the Lockhart Article does not teach or suggest the rejected claims, and that one skilled in the art would not have been motivated to combine the teachings of Lo with the Lockhart Article in a manner so as to render the rejected claims obvious.

Lo teaches a method for identifying, among different probes having unknown sequences, those that exhibit specificity to *N. gonorrhoeae* but not to *N. meningitidis*, wherein the genomic sequences of neither strain are taught by Lo. The probes of Lo are fragments from *N. gonorrhoeae* chromosomal DNA. In Lo, *N. gonorrhoeae* chromosomal DNA is digested into fragments (see, Lo, col. 5, Section A). Each of the fragments is inserted into a vector to form a recombinant molecule (see, Lo, col. 6, Section B). The

recombinant molecule is transformed into a suitable host, e.g., *E. coli* (Lo, col. 6, Sections C and D). The recombinant molecules are amplified (Lo, col. 7, Section E). The recombinant molecules are then screened against *N. gonorrhoeae* and *N. meningitidis* chromosomal DNAs to identify those sequences that are specific for *N. gonorrhoeae* (Lo, col. 8, Section F). The screening is carried out using test dots each consisting of denatured purified chromosomal DNA from either *N. gonorrhoeae* or *N. meningitidis*, i.e., each test dot consists of chromosomal DNA from one strain of *N. gonorrhoeae* or *N. meningitidis* (Lo, col. 8, lines 13-19). Lo does not teach preserving the integrity of chromosomal DNA extracted from the bacteria. Instead, Lo teaches shearing the DNA molecules by passing the molecules through a syringe needle (Lo, col. 22, lines 11-15). Thus, Lo's test dot contains randomly generated fragments of a strain of *N. gonorrhoeae* or *N. meningitidis*. A recombinant molecule is identified if the ratio of its hybridization amount to a test dot containing fragments of chromosomal DNA of a strain of *N. gonorrhoeae* and its hybridization amount to a test dot containing fragments of chromosomal DNA of a strain of *N. meningitidis* is greater than a preset value, e.g., 5 (Lo, col. 10, lines 55-67). Thus, Lo teaches a method of identifying probes that exhibit a ratio of hybridization to a test dot containing fragments of chromosomal DNA of a strain of *N. gonorrhoeae* and hybridization to a test dot containing fragments of chromosomal DNA of a strain of *N. meningitidis* where the sequences of the probes are not known.

The Lockhart Article teaches methods for expression monitoring using pairs of perfect match and mismatch probes on a high density DNA microarray (the Lockhart Article, at page 1676, right column, third paragraph). In the Lockhart Article, a method is disclosed for selecting probes based on the sequence features of the probes. The method involves hybridizing respectively pools of specific cytokine RNAs and complex RNA

populations that do not contain the cytokine RNAs to the 16,000 probe murine cytokine arrays (of more than 16,000 probes) (the Lockhart Article, at page 1680, left column, third paragraph). Data obtained from these experiments were used to extract a set of heuristic rules by a direct analysis of probe behavior as a function of certain sequence features or to train a neural network model (the Lockhart Article, page 1680, left column, third paragraph). The 16,000 probe arrays contain for each target RNA a set of probe pairs, each probe pair containing a perfect-match probe (PM) and a mismatch probe (MM) (the Lockhart Article, page 1676, right column, second and third paragraphs). The abundance of a target RNA is determined based on the difference between the signals of a PM probe and a corresponding MM probe (the Lockhart Article, page 1679, right column). Thus, the Lockhart Article teaches selection of probes according to the difference in binding properties of a PM and a MM, not according to a ratio between the amount of a probe's hybridization to the pool of specific cytokine RNAs and the amount of probe's hybridization to the complex RNA population that did not contain the cytokine RNAs.

**B.1 Claims 27, 29-30, 33-40, 42-54, 59-60, 64-65, 67, 73 and 90-104**

Appellant respectfully submits that neither Lo nor Lockhart, alone or in combination renders the rejected claims obvious. Neither Lo nor Lockhart provide any motivation to a person skilled in the art to combine the teachings of these references as proposed by the Examiner, and thus the combination is improper.

Firstly, as discussed above, the purpose of Lo's method is to identify probes without knowledge of the sequences. This is demonstrated clearly by Lo's method itself: (i) purification and digestion of *N. gonorrhoeae* chromosomal DNA; (ii) formation of a recombinant molecule; (iii) transformation of the recombinant molecule into a suitable host;

(iv) screening of host cells; (v) amplification of the recombinant molecule; (vi) hybridizing each recombinant molecule to test dot consisting of denatured purified chromosomal DNA from a single strain of either *N. gonorrhoeae* or *N. meningitides*; (vii) determining a ratio of hybridization amount to a test dot containing fragments of chromosomal DNA of a strain of *N. gonorrhoeae* and its hybridization amount to a test dot containing fragments of chromosomal DNA of a strain of *N. meningitidis*; and (viii) identifying those recombinant molecules having a ratio greater than a preset value (see also Lo, at col. 5, lines 1-14, for a summary of its method steps). Lo does not teach or suggest using sequence information or determining the sequence of its target and/or probes. Nor does Lo teach or suggest the desirability of using or determining the sequences of its target and/or probes. Instead, its method steps are chosen such that probes can be obtained from the chromosomal DNA of *N. gonorrhoeae* without knowledge of either the target sequence or the probe sequences.

Appellant respectfully submits that Lo does not teach or suggest a method for evaluating a binding property of a polynucleotide probe having a predetermined nucleotide base sequence by determining a ratio of levels of hybridization between a specific pair of samples as defined in independent claims 27, 67, 91 or 93, and their dependent claims.

The Lockhart Article teaches a method of selecting pairs of PM and MM probes having predetermined sequences for measuring the abundances of RNA targets. As discussed above, the Lockhart Article teaches extracting heuristic rules or a neural network model from hybridization data of the 16,000 probe murine cytokine array to a pool of specific cytokine RNAs and to a complex RNA population that did not contain the cytokine RNAs. The Lockhart Article does not teach or suggest comparing directly a probe's hybridization level to the pool of specific cytokine RNAs with the probe's hybridization level to the complex RNA population, much less combining the two hybridization levels of

a probe into a single quantity, e.g., a ratio, and using such a single quantity as a measure of the binding property of the probe. The Lockhart Article does not teach or suggest using probe sequence information in evaluating such a ratio. Nor does the Lockhart Article teach or suggest any advantage or desirability of utilizing sequence information in evaluating such a single quantity measure of binding property of a probe.

Thus, neither Lo or the Lockhart Article provides any motivation to a person skilled in the art for the combination. A person skilled in the art would not be motivated to use sequence information in Lo's method.

Secondly, Lo's method is designed to identify, among different probes having unknown sequences, those that exhibit specificity to *N. gonorrhoeae* as against *N. meningitides*. In Lo's method, a ratio of the level of hybridization of a probe to a sample consisting of randomly generated fragments of chromosomal DNA of a strain of *N. gonorrhoeae*, and the level of hybridization of a probe to a sample consisting of randomly generated fragments of chromosomal DNA of a strain of *N. meningitides*, is used as a measure of the specificity of the probe. Thus, Lo's ratio measures the relative levels of hybridization of the probe to a sample of randomly generated fragments of chromosomal DNA of *N. gonorrhoeae* and to a sample of randomly generated fragments of chromosomal DNA of *N. meningitidis*. Lo does not teach or suggest the pair of samples as specifically defined in claims 27, 67, 91 or 93, much less evaluating a binding property of the probe using such pair of samples.

In contrast to Lo, the presently claimed methods evaluate a binding property of a probe having a predetermined sequence using a defined first sample and second sample, at least one of which has a specific, known sequence composition with respect to the target

sequence and thus to the known sequence of the probe (since the probe comprises a known sequence complementary to at least a hybridizable portion of the target sequence). For example, in the methods claimed in claims 27 and 67, the first sample contains at least 75% polynucleotide molecules that *comprise the target nucleotide sequence* of the probe, while the second sample comprises a plurality of different polynucleotide molecules each comprising a different nucleotide sequence. In the method claimed in claim 91, both samples have a specific, known sequence composition with respect to the target sequence of the probe, and thus to the known sequence of the probe: the first sample contains polynucleotide molecules that *comprise the target sequence* of the probe and a plurality of polynucleotide molecules that do not comprise the target nucleotide sequence; while the second sample comprises a plurality of different polynucleotide molecules each comprising a different nucleotide sequence, which plurality of different polynucleotide molecules do not comprise the target nucleotide sequence. In the method claimed in claim 93, the first sample has a specific, known sequence composition with respect to the target sequence of the probe, and thus to the known sequence of the probe: the first sample contains polynucleotide molecules that *comprise the target nucleotide sequence* of the probe and a plurality of polynucleotide molecules that do not comprise the target nucleotide sequence. As discussed in more detail below, Lo does not teach or suggest evaluating a binding property of a probe using such samples.

Lo does not teach or suggest the first sample of the presently claimed invention. In the presently claimed invention, the first sample "comprises a plurality of polynucleotide molecules *comprising* said target nucleotide sequence" (see, e.g., claim 27, emphasis added). Since "comprising" is construed as "containing at least" (see, e.g., Faber, Robert C., Landis on Mechanics of Patent Claim Drafting, 4th ed., 1998, Practicing Law Institute,

New York, at page II.8), an entire target sequence must be present in at least some polynucleotide molecules in the plurality of polynucleotide molecules in the first sample. In Lo, each probe, i.e., each recombinant molecule, carries a fragment of chromosomal DNA of *N. gonorrhoeae* obtained by, e.g., restriction digestion. A target sequence for such a probe would be a nucleotide sequence that is complementary to at least a hybridizable portion of the recombinant molecule. However, each *N. gonorrhoeae* test dot of Lo consists of randomly generated fragments of chromosomal DNA from a strain of *N. gonorrhoeae*, i.e., fragments produced by shearing (see, Lo, col. 22, lines 16). Lo does not teach or suggest a particular target sequence of its probes, much less that any of these polynucleotide molecules, i.e., DNA fragments, *comprise* the particular target sequence of a probe, i.e., that the fragment encompasses at least a particular target sequence in its entirety.

Because Lo's method does not teach or suggest using the pair of samples of claim 27, 67, 91 or 93, the ratio of Lo does not reflect the hybridization of a probe between such a first sample and a second sample as specified in these claims. In this regard, Appellant respectfully points out that Lo teaches identifying probes that exhibit specificity to *N. gonorrhoeae* as against *N. meningitides*. The ratio of Lo measures the relative levels of hybridization of the probe to a sample of randomly generated fragments of chromosomal DNA of *N. gonorrhoeae* and to a sample of randomly generated fragments of chromosomal DNA of *N. meningitidis*. Thus, Lo's ratio is different from the ratio recited in the claims and the binding property evaluated by the presently claimed method. Thus, even if Lo were to disclose determining the base sequence of its probes (which Lo does not), Lo does not teach or suggest the presently claimed invention, *inter alia*, because of such a difference in what the ratios respectively reflect. Thus, a person skilled in the art would not be motivated to replace Lo's sample with a sample containing polynucleotide molecules that are not

randomly generated, much less with the samples as defined in the rejected claims of the present application. Indeed, it is unclear how Lo's method would still be able to achieve the purpose of Lo with such changed samples if Lo's samples were replaced with the samples as defined in the rejected claims of the present application.

Since a person skilled in the art would not be motivated to modify the samples of Lo's method, the person would not be motivated to combine Lo with the Lockhart Article as put forth by the Examiner. The Lockhart Article also provides no motivation to combine its teachings with Lo. As discussed above, the Lockhart Article teaches a method of using pairs of PM and MM probes having predetermined sequences for measuring the abundances of RNA targets. The Lockhart Article explicitly teaches that it is advantageous to use pairs of PM and MM probes (see, e.g., the Lockhart Article at page 1676, right column, second to last paragraph, and at page 1679, right column, second to last paragraph). A person skilled in the art would understand that the Lockhart Article dissuades one from using a ratio, and thus the Lockhart Article does not motivate a person skilled in the art to combine the Lockhart Article with Lo.

Appellant respectfully submits that the combination of the references put forth by the Examiner is clearly a result of "hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." See, e.g., *In re Fine*, 837 F.2d 1071, 1074 (Fed. Cir. 1988). Using the inventor's disclosure as a blueprint, the Examiner picks and chooses only selective teachings from Lo and the Lockhart Article to defeat the claimed invention. In particular, the Examiner picks from Lo the teachings of determining a ratio of the amount of hybridization of the recombinant molecule to a test dot containing fragments of chromosomal DNA of a strain of *N. gonorrhoeae* and the amount of hybridization of the recombinant molecule to a test dot containing fragments of

chromosomal DNA of a strain of *N. meningitidis*, i.e., Lo's step (vii) discussed above, and from the Lockhart Article the teaching of evaluating probes that have known sequences, to support the obviousness rejection. The Examiner does not provide evidence why a person skilled in the art would have picked such selective teachings from Lo and the Lockhart Article. The Examiner does not consider the context of these teachings and does not evaluate each reference as a whole, but instead, disregards disclosures in the references that diverge from the invention.

The case law is clear that in order to guard against using "hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention," the Examiner must "explain what specific understanding or technological principle within the knowledge of one of ordinary skill in the art would have suggested the combination." *In re Rouffet*, 149 F.3d 1350, 1357 (Fed. Cir. 1998). In the Office Action, in an attempt to show motivation to combine the selected teachings from Lo and the Lockhart Article, the Examiner first contends that "Lockhart et al further teach their method of probe selection, based on sequence information, 'provides a way to use directly the growing body of sequence information for highly parallel experimental investigation .... Simultaneous monitoring of tens of thousands of genes'" (see the Office Action, page 3, middle of second full paragraph). Appellant respectfully points out that, as discussed above, when considered as a whole, a person skilled in the art would understand that the Lockhart Article teaches a method of measuring levels of expression using pairs of PM and MM probes by determining the signal differences between the PM and the MM. The Lockhart Article does not teach or suggest comparing directly a probe's hybridization amount to the pool of specific cytokine RNAs with the probe's hybridization amount to the complex RNA population, much less combining the two hybridization amounts of a probe into a single

quantity, e.g., a ratio, and using such a single quantity as a measure of the binding property of the probe. Thus, the Lockhart Article's teaching provides at most a suggestion of using sequence information in a scheme that utilizes perfect-match and mismatch probe pairs, and does not provide a suggestion of using sequence information in Lo's method. Moreover, the fact that Lockhart teaches that sequence-based probe design is useful for simultaneous monitoring of tens of thousands of gene does not provide the missing motivation to combine the Lockhart Article with a method (Lo's) that is clearly designed so as to avoid using sequence information.

The Examiner also contends that "it would have been obvious to one of ordinary skill in the art ... to apply known sequence analysis for probe selection as taught by Lockhart et al to the probe selection method of Lo et al for the expected benefit of obtaining useful probes based on the growing body of sequence information ...." (see the Office Action, at page 3, third paragraph). Appellant respectfully points out that a vague, non-specific assertion of achieving "the expected benefit of obtaining useful probes based on the growing body of sequence information" is not adequate as evidence of motivation and suggestion. As discussed above, Lo's method is designed to identify probes without using sequence information. The Examiner does not provide evidence regarding how the "known sequence analysis" for selecting perfect-match and mismatch probe pairs taught by the Lockhart Article would benefit such a probe selection method of Lo. The Examiner does not provide evidence regarding how to modify Lo's samples according to the probe sequence. As discussed above, Lo's samples, i.e. test dots, consist of randomly generated fragments of chromosomal DNA of a strain of *N. gonorrhoeae* or a strain of *N. meningitides*. The base sequences of different polynucleotide molecules as well as their relative proportions in the samples are unknown. Lo's method identifies probes that exhibit

specificity when using such samples of randomly generated chromosomal DNA. The Examiner does not provide evidence regarding why simply knowing the probe sequence without more would allow Lo's method to achieve "the expected benefit of obtaining useful probes."

In addition, the Examiner does not provide evidence regarding how to apply known sequence analysis to Lo's method to arrive at the presently claimed methods. The Examiner does not provide evidence regarding which step(s) of Lo may benefit from using sequence information. The Examiner does not provide evidence regarding which sequence or sequences are useful. In this regard, Lo uses 6 strains of *N. gonorrhoeae* and 6 strains of *N. meningitides*, and screened 3,000 probes (see, Lo, col. 13, lines 13-20). The Examiner does not provide evidence regarding which sequences of the genomic sequence(s) of which strain or strains of *N. gonorrhoeae* should be used in the probes and the samples. The Examiner does not provide evidence regarding where or how to obtain any such sequences. As discussed above, without using samples containing polynucleotide molecules having a specified relationship to the probe sequences as set forth in the first and/or second sample in the presently claimed methods, the ratio of probe hybridization levels does not correspond to the binding property evaluated by the presently claimed methods. Thus, the Examiner's contention is nothing more than "[b]road conclusory statements regarding the teaching of multiple references," which, standing alone, "are not 'evidence.'" *In re Dembiczak*, 175 F.3d 994, 999 (Fed. Cir. 1999).

The Examiner fails to evaluate each reference as a whole. As discussed above, the Examiner chooses Lo's teaching of determining a ratio of the amount of hybridization of the recombinant molecule to a test dot containing fragments of chromosomal DNA of a strain of *N. gonorrhoeae* and the amount of hybridization of the recombinant molecule to a test

dot containing fragments of chromosomal DNA of a strain of *N. meningitidis*, i.e., Lo's step (vii) discussed above, but disregards Lo's method steps that do not use or rely on sequence information, e.g., Lo's steps (i)-(vi). On the other hand, the Examiner chooses the Lockhart Article's teaching of evaluating probes that have known sequences, but disregards the parts of the Lockhart Article's teachings of using pairs of PM and MM probes for determining the signal differences between PM probes and MM probes. Appellant respectfully submits that when Lo is evaluated as a whole, it would be clear to a person skilled in the art that Lo does not teach or suggest using sequence information in its method, whereas when the Lockhart Article is evaluated as a whole, it would be clear to a person skilled in the art that the Lockhart Article does not teach using sequence information in determining a ratio as a measure of a binding property of the probe. In *In re Wesslau*, the court has held that "[i]t is *impermissible* within the framework of a Section 103 rejection *to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary to the full appreciation of what the reference fairly suggests* to one of ordinary skill in the art." *In re Wesslau*, 353 F.2d 238, 241 (C.C.P.A. 1965) (emphasis added).

In the Office Action, the Examiner also contends that the obviousness conclusion based on a combination of Lo and the Lockhart Article is proper, citing *In re McLaughlin*, 170 U.S.P.Q. 209 (C.C.P.A. 1971). The Examiner cited

> [a]ny judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning, but so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made and does not include knowledge gleaned only from applicant's disclosure, such a reconstruction is proper.

*In re McLaughlin*, 170 U.S.P.Q. 209, 212 (C.C.P.A. 1971). Appellant respectfully points out that, as discussed above, the Examiner's reconstruction in the present case was achieved

by picking and choosing from Lo the teachings of hybridizing each recombinant molecule to a test dot of a strain of *N. gonorrhoeae* and a test dot of a strain of *N. meningitidis* and determining a ratio of the respective levels of hybridization, i.e., Lo's step (vii) discussed above, and from the Lockhart Article the teaching of evaluating probes that have known sequences, while disregarding disclosures in both references that diverge from the invention. The Examiner does not provide evidence why a person skilled in the art, without knowledge of the presently claimed invention, would have picked such selective teachings from Lo and the Lockhart Article. Therefore, the Examiner's reconstruction includes knowledge gleaned only from the presently claimed invention. Such a reconstruction is not sanctioned by *In re McLaughlin*.

Appellant further respectfully points out that the Examiner characterizes various teachings of Lo incorrectly and inconsistently in support of the § 103 rejection. For example, the Examiner contends that the chromosomal DNA of *N. gonorrhoeae* is the target (see the Office Action at page 3, last paragraph, bridging to page 4). The Examiner also contends that Lo teaches a first sample which comprises a plurality of molecules comprising the target chromosomal DNA from a strain of *N. gonorrhoeae* (see the Office Action at page 3, line 4). The Examiner appears to contend that Lo's first sample contains a plurality of intact chromosomal DNA molecules of *N. gonorrhoeae* which comprises the entire genomic DNA of *N. gonorrhoeae*. The Examiner's reasoning appears to be that since each of such intact chromosomal DNA molecules would comprise any target sequences, Lo teaches the first sample of the presently claimed invention. However, as discussed above, Lo does not teach such a sample. Lo does not teach or suggest that its test dots contains intact chromosomal DNA molecules, nor that there is any desirability of using test dots

having intact chromosomal DNA molecules. To the contrary, Lo teaches shearing chromosomal DNAs in preparing its test dots (see, e.g., Lo, col. 22, lines 11-15).

The Examiner also contends that Lo teaches "a second sample which comprises a plurality of different polynucleotides (i.e. chromosomal DNA from *N. meningitidis* ... and chromosomal DNA from *N. gonorrhoeae*...)" (see the Office Action at page 2, last three lines). The meaning of this contention is unclear. Because the second sample of the presently claimed invention comprises a plurality of different molecules, Appellant assumes that the Examiner was contending that Lo teaches a second sample which comprises chromosomal DNA from a plurality of different strains of *N. gonorrhoeae* and *N. meningitidis*, "i.e., a second sample comprising chromosomal DNA from *N. gonorrhoeae*, strains 53415, 53416, 53417, 53418 *and* 53419 *and* chromosomal DNA from *N. gonorrhoeae*, 53420, 53421, 53422, 53423, 53424, 53425" (see the Office Action at page 4, last paragraph, bridging to page 5, emphasis added). As discussed above, Lo teaches that each individual test dot, i.e., each sample, consists of DNA molecules from one strain of either *N. gonorrhoeae* or *N. meningitides* (see, e.g., Lo, col. 8, lines 13-19). Lo does not teach a test dot that comprises a mixture of chromosomal DNA from two or more different strains. Lo teaches using hybridization to each individual dot in the screening of the recombinant molecules. Thus, Lo does not teach a second sample comprising "chromosomal DNA from *N. N. meningitides*, strains 53415, 53416, 53417, 53418 and 53419 and chromosomal DNA from *N. gonorrhoeae*, 53420, 53421, 53422, 53423, 53424, 53425."

With respect to claims 91 and 93, the Examiner contends that Lo teaches a first sample comprising a plurality of molecules "comprising the target chromosomal non-homologous DNA and a plurality of molecules that do not comprise the target i.e.

homologous DNA" (see the Office Action, page 8, third full paragraph). The Examiner cites Lo at col. 3, lines 10-30 in support of the contention. Appellant first respectfully submits that the Examiner thereby appears to construe the target sequence as the sequences in the genome of *N. gonorrhoeae* that are non-homologous to sequences in the genome of *N. meningitides*. This is contradictory to the Examiner's contention with respect to claims 27 and 67 that the chromosomal DNA of *N. gonorrhoeae* is the target. This is also contradictory to the Examiner's contention with respect to claims 29, 37, 39, 42, 92 and 94 that the target sequence is a sequence from a gene (see below Section B.3). Because Lo's test dots contain randomly generated fragments of *N. gonorrhoeae* DNA, Lo cannot teach or suggest that its test dots contain a plurality of polynucleotide molecules *comprising* any particular non-homologous sequence. Appellant also respectfully submits that the cited passage of Lo is a passage in the background section of Lo which discusses various problems in the art with respect to finding probes that can be used to distinguish *N. gonorrhoeae* from *N. meningitidis*. The passage teaches that because there is a higher sequence homology between the sequence of any one strain of *N. gonorrhoeae* and the sum total sequences of numerous strains of *N. meningitidis*, the percentage of sequences in the genome of *N. gonorrhoeae* that is nonhomologous to the sum total sequence of numerous strains of *N. meningitides* may be even smaller than to an individual stran of *N. meningitides*, and that, among such sequences, discrete sequences that are nonhomologous to the sum total sequence of numerous strains of *N. meningitidis* may or may not exist. The cited passage is a discussion of information purportedly in the art in the Background section, and does not teach specifically the polynucleotide compositions of Lo's samples, i.e., Lo's test dots.

Therefore, for the above reasons, Appellant respectfully submits that Lo in view of the Lockhart Article does not render claims 27, 67, 91 and 93 obvious.

With respect to the dependent claims, Appellant first respectfully submits that because independent claims 27, 67, 91 and 93 are not rendered obvious by Lo in view of the Lockhart Article, the dependent claims are also nonobvious. The Federal Circuit has held that "[d]ependent claims are nonobvious under section 103 if the independent claims from which they depend are nonobvious." *In re Fine*, 837 F.2d 1071, 1076 (Fed. Cir. 1988). Appellant respectfully submits that Lo and the Lockhart Article, alone or in combination, do not render claims 27, 29-30, 33-40, 42-54, 59-67, 73-75, 84-85 and 90-104 obvious, and the rejection of these claims under 35 U.S.C. § 103(a) based on Lo in view of the Lockhart Article should be withdrawn.

**B.2. Claims 27, 29-30, 33-40, 42-67, 73-75, 84-85, and 90**

With respect to claims 27 and 67, and dependent claims 29-30, 33-40, 42-66, 73-75, 84-85, and 90, Appellant respectfully submits that these claims have the additional limitation that at least 75% of the polynucleotide molecules in the first sample are polynucleotide molecules comprising the target nucleotide sequence. Lo does not teach or suggest a first sample that contains at least 75% polynucleotide molecules that comprise a target sequence of a probe. As discussed above, Lo teaches a sample that contains randomly generated fragments. A person skilled in the art would understand that in such a sample, the amount of polynucleotide molecules that comprise any particular target sequence is not defined. Since the test dot contains randomly generated fragments, it is unreasonable to expect that at least 75% of the polynucleotide molecules in the test dot would contain the same target sequence. Thus, Lo cannot teach or suggest a first sample

that contains at least 75% polynucleotide molecules that comprise a particular target sequence.

As discussed above, the Lockhart Article is concerned with finding a pair of PM and MM probes that offers the optimal signal difference for determining the amount of hybridization of the target in a sample, and is not concerned with comparing directly a probe's hybridization amount to a first sample with the probe's hybridization amount to a second sample, much less combining the two hybridization amounts of a probe into a single quantity, e.g., a ratio, and using such a single quantity as a measure of the binding property of the probe. Thus, irrespective of whether the Lockhart Article teaches probes that target nucleotide sequences from a gene or gene transcript, the Lockhart Article does not provide motivation to a person skilled in the art to modify the method of Lo in such a way as to render the presently claimed invention of claims 27, 29-30, 33-40, 42-67, 73-75, 84-85, and 90 obvious.

Thus, Lo and the Lockhart Article do not teach or suggest a method of evaluating a binding property of a probe using a first sample in which at least 75% of the polynucleotide molecules are polynucleotide molecules comprising the target nucleotide sequence, and claims 27, 29-30, 33-40, 42-67, 73-75, 84-85, and 90 (in part) are separately patentable.

**B.3. Claims 29, 37, 39, 42, 92 and 94**

With respect to claims 29, 37, 39, 42, 92 and 94, Appellant respectfully submits that these claims have the additional limitation that the target polynucleotide sequence is a nucleotide sequence from a gene or gene transcript of a cell or organism or an mRNA, cDNA or cRNA derived therefrom (claim 29) or a nucleotide sequence from a gene or gene transcript of a cell or organism (claims 37, 39, 42, 92 and 94). Thus, the methods as

claimed by these claims evaluate a binding property of a probe having a predetermined sequence that is complementary to at least a hybridizable portion of a target sequence of a gene or gene transcript of a cell or organism (or an mRNA, cDNA or cRNA derived therefrom for claim 29) using a first sample that comprises polynucleotide molecules comprising such a target sequence. In contrast, as discussed above, Lo teaches that its probes are fragments of chromosomal DNA of *N. gonorrhoeae*. As is well known in the art, chromosomal DNA of an organism contains both sequences corresponding to genes ("gene sequences") and sequences not corresponding to genes ("non-gene sequences"). Lo teaches that its probes are generated without consideration as to whether they contain gene or non-gene sequences, e.g., by restriction digestion of the chromosomal DNA. Thus, Lo does not teach or suggest probes that target genes. Nor is it inevitable that its probes would target genes, since, as discussed above, Lo's probes are generated by restriction digestion of the chromosomal DNA, and it is unknown whether such a fragment generated by restriction digestion contains gene sequences or whether it contains non-gene sequences. Appellant notes that even if it can be expected that some fragments would probably contain sequences of genes, it is not known for each particular probe whether it contains sequences of a gene and thus targets a gene. Thus, it is not inherent in the disclosure of Lo that any particular probe target a gene, since inherency requires inevitability. See *Continental Can Co. v. Monsanto Co.*, 948 F.2d 1264, 1269 (Fed. Cir. 1991), quoting *In re Oelrich*, 666 F.2d 578 (C.C.P.A. 1981).

The purpose of Lo's method is to identify fragments from *N. gonorrhoeae* chromosomal DNA which exhibit specificity to *N. gonorrhoeae* chromosomal DNA relative to *N. meningitidis* chromosomal DNA. A person skilled in the art would understand that for such a purpose, whether the probes' target sequences are from a sequence of a gene or from

a non-gene sequence is irrelevant. Thus, Lo does not motivate or suggest including steps to ensure that the probes are such that their target sequences are sequences of genes. A person skilled in the art would not be motivated to modify Lo's method to evaluate probes that target genes.

In the Office Action, with respect to claim 29, the Examiner contends that "Lo et al disclose the method in which the target in the first sample is a sequence of a gene from an organism i.e. *N. gonorrhoeae* chromosomal DNA (column 4, line 44 through column 5, line 65)" (the Office Action, page 3, last paragraph). Appellant respectfully points out that the passages of Lo cited by the Examiner do not teach that the target in the first sample is a sequence of a gene from an organism i.e. *N. gonorrhoeae*. Appellant notes that the cited passages does not contain descriptions of Lo's samples (which are described in col. 8, section F of Lo).

With respect to claims 37, 39, 42, 92 and 94, the Examiner first merely reiterates the contention applied to claim 27 (see , the Office Action, page 4, last paragraph) and then contends that Lo teaches a method for screening closely related samples that are specific for a "genetically distinct group" (see the Office Action, page 4, last paragraph through page 5, second full paragraph). Neither contentions address the additional limitation in the claimed methods that the target polynucleotide sequence is a nucleotide sequence from a gene or gene transcript of a cell or organism, or of an mRNA, cDNA or cRNA derived therefrom. Appellant respectfully points out that the genetically distinct group in Lo means the bacterial species, i.e., *N. gonorrhoeae*, for which Lo's probes are specific. Lo does not teach or suggest that its method is useful for wild-type vs. mutant. Appellant further respectfully points out that the Examiner's contention is clearly hindsight based reconstruction.

As discussed above, the Lockhart Article is concerned with finding a pair of PM and MM probes that offers the optimal signal difference for determining the amount of hybridization of the target in a sample, and is not concerned with comparing directly a probe's hybridization amount to a first sample with the probe's hybridization amount to a second sample, much less combining the two hybridization amounts of a probe into a single quantity, e.g., a ratio, and using such a single quantity as a measure of the binding property of the probe. Thus, irrespective of whether the Lockhart Article teaches probes that target nucleotide sequences from a gene or gene transcript, the Lockhart Article does not provide motivation to a person skilled in the art to modify the method of Lo in such a way as to render the presently claimed invention of claims 37, 39, 42, 92 and 94 obvious.

Thus, Lo and the Lockhart Article do not teach or suggest a method of evaluating a binding property of a probe whose target sequence is from a gene or gene transcript (claims 37, 39, 42, 92 and 94) or whose target sequence is from a gene or gene transcript or an mRNA, cDNA or cRNA derived therefrom (claim 29) using a first sample that comprises a plurality of polynucleotide molecules that comprise the target sequence, and claims 29, 37, 39, 42, 92 and 94 are separately patentable.

**B.4. <u>Claims 37, 39, 42, 92 and 94</u>**

In addition, claims 37 and 42 have the limitation that the second sample comprises a polynucleotide sample from a deletion mutant of the cell or organism which does not express the gene or gene transcript. Claims 39, 92 and 94 have the limitation that the second sample comprises a polynucleotide sample from a wild-type strain of the cell or organism that expresses the gene or gene transcript.

Thus, in the method of claims 37, 39, 42, 92 and 94, the first and second samples

contain polynucleotide molecules from the same organism, i.e., a wild-type strain or deletion mutant of the same organism. In contrast, Lo teaches first and second samples containing polynucleotide molecules from different organisms, i.e., *N. gonorrhoeae* and *N. meningitidis*. As discussed above, the Lockhart Article cannot properly be used to remedy this deficiency. Thus, claims 37, 39, 42, 92 and 94 are separately patentable.

**B.5. Claims 36, 40 and 91-92**

With respect to claims 36, 40, and 91-92, these claims have the additional limitation that the different polynucleotide molecules in the second sample do not comprise the target nucleotide sequence. Thus, the methods as claimed by these claims evaluate a binding property of a probe having a predetermined sequence by determining a ratio of hybridization of the probe to a first sample that comprises polynucleotide molecules comprising the target sequence sequence and a second sample that does not comprise the target sequence. In contrast, because none of its probes' sequences are known, Lo cannot teach or suggest that any of the polynucleotide molecules in its second sample, i.e., test dots of *N. meningitidis*, do not comprise any particular sequence, much less the target nucleotide sequence. Appellant also respectfully points out that Lo's method is designed to identify probes that are specific to *N. gonorrhoeae* but not *N. meningitidis*, two bacteria having a high degree of DNA homology (see, e.g., Lo at col. 2, lines 58-61). A person skilled in the art would understand that if the second sample is known to not comprise the target sequence, then there is no need to perform Lo's method. Thus, a person skilled in the art would not be motivated to modify Lo's method such that its second sample does not comprise the target of the probe.

In the Office Action, with respect to claims 36, 40 and 91, the Examiner contends

that Lo discloses a method in which "the second sample does not comprise the target (i.e., chromosomal DNA from *N. gonorrhoeae*) but instead comprises chromosomal DNA from *N. meningitidis*" (the Office Action, page 4, second to last paragraph; page 5 last line through page 6, first two lines). Appellant respectfully points out that in the presently claimed invention of claims 36, 40, 91 and 92, the second sample comprises a plurality of different polynucleotide molecules and does not comprise the target sequence. A teaching that *the second sample* does not comprise chromosomal DNA from *N. gonorrhoeae* but comprises chromosomal DNA from *N. meningitidis* does not teach or suggest whether the polynucleotide molecules comprise the same target sequence or not.

As discussed above, the Lockhart Article is concerned with finding a pair of PM and MM probes that offers the optimal signal difference for determining the amount of hybridization of the target in a sample, and is not concerned with comparing directly a probe's hybridization amount to a first sample with the probe's hybridization amount to a second sample, much less combining the two hybridization amounts of a probe into a single quantity, e.g., a ratio, and using such a single quantity as a measure of the binding property of the probe. Thus, irrespective of whether the Lockhart Article teaches using a reference sample that does not contain target sequences, the Lockhart Article does not provide motivation to a person skilled in the art to modify the method of Lo in such a way as to render the presently claimed invention of claims 36, 40, 85, 91 and 92 obvious.

Thus, Lo and the Lockhart Article do not teach or suggest a method of evaluating a ratio of hybridization of a probe between a first sample that comprises a plurality of polynucleotide molecules that comprise the target sequence and a second sample that contains different polynucleotide molecules that do not comprise the target nucleotide sequence, and claims 36, 40, 85 and 91-92 are separately patentable.

## B.6. Claim 38

With respect to claim 38, this claim has the additional limitation that the different polynucleotide molecules in the second sample comprise both polynucleotide molecules comprising the target nucleotide sequence and a plurality of different polynucleotide molecules that does not comprise the target nucleotide sequence. Thus, the method as claimed by claim 38 evaluates a binding property of a probe having a predetermined sequence by determining a ratio of hybridization of the probe to a first sample that comprises polynucleotide molecules comprising the target sequence sequence and a second sample that comprises both polynucleotide molecules that comprise the target sequence and a plurality of different polynucleotide molecules not comprising the target sequence. In contrast, because none of its probes' sequences are known, Lo cannot teach or suggest that any of the polynucleotide molecules in its second sample, i.e., test dots of *N. meningitidis*, comprise both polynucleotide molecules that comprise the target sequence and a plurality of different polynucleotide molecules not comprising the target sequence. Appellant also respectfully points out that Lo's method is designed to identify probes that are specific to *N. gonorrhoeae* but not *N. meningitidis*, two bacteria having a high degree of DNA homology (see, e.g., Lo at col. 2, lines 58-61). A person skilled in the art would not be motivated to modify Lo's method such that its second sample comprises both polynucleotide molecules that comprise the target sequence and polynucleotide molecules that do not comprise the target sequence of the probe.

In the Office Action, the Examiner contends that Lo discloses a method in which "the second sample comprises polynucleotides comprising the target and a plurality of different molecules comprising a different sequence, not the target. Lo et al teach the second sample comprises chromosomal DNA from different *N. meningitidis* strains and

from *N. gonorrhoeae* strains" (the Office Action, page 5). Appellant respectfully points out that, as discussed above, Lo's first sample consists of chromosomal DNA from *N. gonorrhoeae* while Lo's second sample consists of chromosomal DNA from *N. meningitidis*. Lo does not teach any sample that "comprises chromosomal DNA from different *N. meningitidis* strains and from *N. gonorrhoeae* strains." For example, Lo clearly teaches that "[e]ach of the test dots consists of denatured purified chromosomal DNA isolated from one of the following strains of *Neisseria gonorrhoeae* and DNA from *Neisseria meningitidis* ..." (see, Lo, column 8, lines 13-19).

As discussed above, the Lockhart Article is concerned with finding a pair of PM and MM probes that offers the optimal signal difference for determining the amount of hybridization of the target in a sample, and is not concerned with comparing directly a probe's hybridization amount to a first sample with the probe's hybridization amount to a second sample, much less combining the two hybridization amounts of a probe into a single quantity, e.g., a ratio, and using such a single quantity as a measure of the binding property of the probe. Thus, irrespective of whether the Lockhart Article teaches using a reference sample that does not contain target sequences, the Lockhart Article does not provide motivation to a person skilled in the art to modify the method of Lo in such a way as to render the presently claimed invention of claim 38 obvious.

Thus, Lo and the Lockhart Article do not teach or suggest a method of evaluating a ratio of hybridization of a probe between a first sample and a second sample, wherein the second sample contains polynucleotide molecules comprising the target nucleotide sequence and a plurality of different polynucleotide molecules that does not comprise the target nucleotide sequence, and claim 38 is separately patentable.

### B.7. Claims 43-54 and 95-104

With respect to claims 43-54 and 95-104, Appellant respectfully submits that these claims each have an additional limitation specifying a specific relative abundance of polynucleotides comprising the target sequence and polynucleotides not comprising the target sequence, between the first sample and the second sample. For example, the amount of polynucleotide molecules in the first sample comprising the target nucleotide sequence differs from the amount of polynucleotide molecules in the second sample comprising the target nucleotide sequence by at least a factor of two (claims 43 and 95), at least a factor of four (claims 44 and 96), at least a factor of eight (claims 45 and 97), at least a factor of twenty (claims 46 and 98), or at least a factor of 100 (claims 47 and 99); or each polynucleotide molecule that does not comprise the target nucleotide sequence in the first sample is present in the second sample in an amount that differs from the amount of said polynucleotide molecule in the first sample by no more than a factor of 100 (claim 48), no more than a factor of 10 (claim 49), no more than 50% (claims 50 and 100); or the mean abundance of the polynucleotide molecules that do not comprise the target nucleotide sequence in the first sample differs from the mean abundance of the different polynucleotide molecules that do not comprise the target nucleotide sequence in the plurality of different polynucleotide molecules of the second sample by no more than a factor of two (claims 51 and 101), no more than 50% (claims 52 and 102), no more than 10% (claims 53 and 103), no more than 1% (claims 54 and 104). Because none of its probes' sequences are known, Lo cannot teach or suggest such a specific correlation in amounts of polynucleotides (either polynucleotides comprising the target sequence or polynucleotides not comprising the target sequence) between the first sample and the second sample.

In the Office Action (see the Office Action at page 6, second paragraph), with respect to claims 43-47 and 95-99, the Examiner contends that in Lo's method the first sample spots have 500 nanograms of chromosomal DNA and the second sample spots have 5 picograms of chromosomal DNA, thereby providing amounts of polynucleotides differing by at least a factor of 100, citing Lo, column 11, lines 29-42. With respect to claims 48-54 and 100-104, the Examiner contends that Lo teaches a probe evaluation method in which "the amount/abundance of polynucleotide in the first sample is the same as the amount/abundance in the second sample ... therefore differs by no more than a factor of two ... no more than 1%," citing Lo, column 11, lines 29-42 (see the Office Action page 6, third paragraph, and page 11, second paragraph). Appellant respectfully points out that in the cited section, Lo teaches six serially diluted chromosomal DNA samples for each strain, i.e., six samples of chromosomal DNA of a strain of *N. gonorrhoeae* or six samples of chromosomal DNA of a strain of *N. meningitidis*. However, because each sample consists of randomly fragmented chromosomal DNA, a teaching of the total DNA amount in a test dot does not teach or suggest anything about the amount of any particular polynucleotide molecules, e.g., molecules having a specified sequence or molecules not having a specified sequence, in the test dot. Thus, Lo cannot teach or suggest the relative amounts of molecules having a specified sequence (or not having a specific sequence) between any sample of *N. gonorrhoeae* and any sample of *N. meningitidis*, regardless of the respective dilution factors. For example, Lo cannot teach or suggest that the ratio of the relative amounts of molecules having a specified sequence (or not having a specific sequence) between the sample having 500 nanograms of *N. gonorrhoeae* chromosomal DNA and the sample having 500 nanograms of *N. meningitidis* chromosomal DNA, much less the relative amounts of molecules having a specified sequence between the sample having 500

nanograms of *N. gonorrhoeae* chromosomal DNA and the sample having 5 picograms of *N. meningitidis* chromosomal DNA.

The Examiner also contends that the open claim language "comprising" encompasses the first sample having polynucleotides not having the target i.e. *N. meningitidis* (see the Office Action page 6, second paragraph, and page 10, last paragraph). Appellant respectfully points out that indeed the claim language encompasses a first sample having polynucleotides not having the target (indeed, claim 43 part (a) specifies as much). However, this is irrelevant to the substance of the Examiner's rejection. Lo does not render these claims obvious, since it does not provide a suggestion to a person skilled in the art of such a first sample. Without such a suggestion, the claims are nonobvious.

As discussed above, the Lockhart Article is concerned with finding a pair of PM and MM probes that offers the optimal signal difference for determining the amount of hybridization of the target in a sample, and is not concerned with comparing directly a probe's hybridization amount to a first sample with the probe's hybridization amount to a second sample, much less combining the two hybridization amounts of a probe into a single quantity, e.g., a ratio, and using such a single quantity as a measure of the binding property of the probe. Thus, irrespective of whether the Lockhart Article teaches using a reference sample that does not contain target sequences, the Lockhart Article does not provide motivation to a person skilled in the art to modify the method of Lo in such a way as to render the presently claimed invention of claims 43-54 and 95-104 obvious.

Thus, Lo and Lockhart does not teach or suggest a method of evaluating a ratio of hybridization of a probe between a first sample that comprises a plurality of polynucleotide molecules that comprise the target sequence and a plurality of polynucleotide molecules that

do not comprise the target and a second sample that contains different polynucleotide molecules as specified in claims 43-54 and 95-104, and these claims are separately patentable.

In view of the foregoing, Appellant respectfully submits that Lo and the Lockhart Article, alone or in combination, do not render claims 27-30, 33-36, 44-47, 59-68, 73-75, 90, 91 and 93 obvious. The rejection is in error, and should be reversed.

**C. The Rejection of Claims 61-63, 66, 74-75 and 84-85 over Lo In View of the Lockhart Article In Further view of the Lockhart Patent Is Erroneous and Should Be Reversed**

With respect to the second issue on appeal, claims 61-63, 66, 74-75 and 84-85 are rejected as being obvious over U.S. Patent No. 4,900,659 ("Lo") in view of Lockhart et al., Nature Biotechnology 14:1675-1680 ("the Lockhart Article") as applied to claims 27 and 67, and in further view of Lockhart et al., U.S. Patent No. 6,344,316 B1 ("the Lockhart Patent"). The Examiner contends that while Lo does not teach differentially labeling the probes with fluorescence labels and does not teach attaching probes to different locations on a microarray, Lockhart teaches both differential labeling with fluorescence labels and attaching probes to microarrays (see the Office Action at page 11, last full paragraph to page 12 second paragraph). The Examiner contends that "[i]t would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to apply the probe array of Lockhart et al to the probe analysis method of Lo et al for the obvious benefit of optimizing a high-density array of probes as desired by Lockhart et al" (see the Office Action at page 12, last sentence of the first paragraph). The Examiner also contends that it would have been obvious to differentially label the polynucleotides of Lo to provide

independent analysis of simultaneously hybridized polynucleotides as taught by the Lockhart Patent (see the Office Action at page 13, first paragraph). Appellant respectfully submits that Lo in combination with the Lockhart Article and in further combination with the Lockhart Patent does not render the rejected claims obvious.

Lo and the Lockhart Article have been described above in Section B. The Lockhart Patent teaches methods for identifying differences in nucleic acid abundances (e.g., expression levels) between two or more samples using high density DNA microarrays. In the Lockhart Patent, a method of optimizing a set of probes for detection of a particular gene is disclosed. The probe optimization method involves first hybridizing the probes with their target nucleic acids alone and then hybridizing the probes with a high complexity, high concentration nucleic acid sample that does not contain the targets complementary to the probes (the Lockhart Patent, column 36, lines 30-36), and selecting those probes that show a strong hybridization signal with their target and little or no cross-hybridization with the high complexity sample as preferred probes for use in the high density arrays (the Lockhart Patent, column 36, lines 44-47). The Lockhart Patent then provides a detailed explanation of how the foregoing is accomplished. For selection of probes showing a strong hybridization signal with their target with the high complexity sample, the Lockhart Patent teaches that the probes are hybridized to a sample containing target nucleic acids having subsequences complementary to the oligonucleotide probes, and those probes are selected for which the difference in hybridization intensity between the perfect-match probes and their respective mismatch controls exceeds a threshold hybridization intensity (see, e.g., the Lockhart Patent col. 37, lines 1-12). For selection of probes showing little or no cross-hybridization, the Lockhart Patent teaches that the probes can be hybridized with a nucleic acid sample that is not expected to contain sequences complementary to the probes, and

those probes are selected for which both the probes and their mismatch controls show hybridization intensities below a threshold value (see, e.g., the Lockhart Patent col. 37, lines 13-27). Thus, in the Lockhart Patent, selection of probes that show a strong hybridization signal with their target and little or no cross-hybridization is achieved by evaluating a probe according to its ability to hybridize to the target sample, and comparing this ability to a threshold, and separately, evaluating the probe according to its ability to hybridize to the non-target sample, and comparing this latter ability to a threshold. The Lockhart Patent does not teach or suggest comparing directly the hybridization signal and cross-hybridization signal of the same probe, much less combining the hybridization signal and cross-hybridization signal of the same probe into a single quantity, e.g., a ratio, and using such a single quantity as a measure of the binding property of the probe.

**C.1. <u>Claims 61-63, 66, 74-75 and 84-85</u>**

Appellant first respectfully submits that, for the reasons discussed above, independent claims 27 and 67 are not rendered obvious by Lo in view of the Lockhart Article. Similar to the Lockhart Article, as discussed above the Lockhart Patent also is concerned with finding a pair of PM and MM probes that offers the optimal signal difference for determining the amount of hybridization of the target in a sample, and is not concerned with comparing directly a probe's hybridization amount to a first sample with the probe's hybridization amount to a second sample. In the Office Action, the Lockhart Patent is cited for its teaching of differential labeling with fluorescence labels and attaching probes to microarrays (see the Office Action at page 11, last full paragraph to page 12 second paragraph). Thus, the Lockhart Patent does not add what is missing in Lo and the Lockhart Article with respect to independent claims 27 and 67. As discussed above, the Federal Circuit has held that "[d]ependent claims are nonobvious under section 103 if the

independent claims from which they depend are nonobvious." *In re Fine*, 837 F.2d 1071, 1076 (Fed. Cir. 1988). Thus, Appellant respectfully submits that Lo and the Lockhart Article in further view of the Lockhart Patent do not render claims 61-63, 66, 74-75 and 84-85 obvious.

With respect to the Examiner's contention that it would have been obvious to a person skilled in the art to apply the probe array of the Lockhart Patent to the probe analysis method of Lo for the obvious benefit of optimizing a high-density array of probes as desired by the Lockhart Patent (see the Office Action at page 12, last sentence of the first paragraph), Appellant respectfully submits that, as discussed above, the Lockhart Patent teaches optimizing a microarray containing pairs of PM and MM probes for determining the signal differences of PMs and MMs. The Lockhart Patent does not teach or suggest comparing directly a probe's level of hybridization to a first sample with the probe's level of hybridization to a second sample, much less determining a ratio of the levels of hybridization and using such a ratio as a measure of the binding property of the probe. The Lockhart Patent does not provide motivation to a person skilled in the art to modify its method according to Lo. Thus, the Lockhart Patent does not suggest any desire to optimize its probe array using the probe analysis method of Lo.

With respect to the Examiner's contention that it would have been obvious to a person skilled in the art to differentially label with fluorescent labels the polynucleotides of Lo to provide independent analysis of simultaneously hybridized polynucleotides as taught by the Lockhart Patent (see the Office Action at page 11, last full paragraph and the paragraph bridging to page 12), Appellant first respectfully points out that it is unclear as to what the Examiner intends to mean by the contention. Appellant assumes that the Examiner means to contend that it would be obvious to differentially label Lo's test dots with

fluorescent labels. Appellant respectfully points out that, in Lo's method, polynucleotides in samples are fixed on a surface, and hybridization was detected by labeled probes. A person skilled in the art would understand that there is no need to label the polynucleotides in Lo's samples, much less to label them differentially. In addition, a person skilled in the art would also understand that, in such a method, hybridization of a probe to different samples can be detected without the need of differential labeling, because the different samples, i.e., different test dots, are spatially addressable. Thus, a person skilled in the art would not be motivated to differentially label with fluorescent labels the polynucleotides of Lo.

### C.2. Claim 75

With respect to claim 75, Appellant respectfully submits that claim 75 has the additional limitation that the first sample comprises two or more different polynucleotide molecules, wherein none of the two or more different polynucleotide molecules hybridizes or cross-hybridizes to a probe that also hybridizes or cross-hybridizes to another one of the two or more different polynucleotide molecules. Since Lo does not know the base sequence of any of its probes nor the sequences of nucleic acid molecules in its test dots, Lo cannot teach or suggest a method using such a sample. Both the Lockhart Article and the Lockhart Patent are concerned with finding a pair of PM and MM probes which offers the optimal signal difference for determining the amount of hybridization of the target in a sample, and are not concerned with comparing directly a probe's level of hybridization to a first sample with the probe's level of hybridization to a second sample, much less determining a ratio of the levels of hybridization and using such a ratio as a measure of the binding property of the probe. Thus, irrespective of whether the Lockhart Article or the Lockhart Patent teaches differential labeling with fluorescence labels or attaching probes to microarrays, they do not

provide motivation to a person skilled in the art to modify the method of Lo in such a way as to render the presently claimed invention of claim 75 obvious.

Thus, Lo, the Lockhart Article and the Lockhart Patent do not teach or suggest a method of evaluating a ratio of hybridization of a probe in which the first sample comprises two or more different polynucleotide molecules, wherein none of the two or more different polynucleotide molecules hybridizes or cross-hybridizes to a probe that also hybridizes or cross-hybridizes to another one of the two or more different polynucleotide molecules, and claim 75 is separately patentable.

### C.3. Claim 85

With respect to claim 85, Appellant respectfully submits that the claim has the additional limitation that the second sample lacks polynucleotide molecules of the first sample. Thus, the method as claimed by the claim evaluates a binding property of a probe having a predetermined sequence by determining a ratio of hybridization of the probe to a first sample that comprises polynucleotide molecules comprising the target sequence and a second sample that does not comprise the molecules present in the first sample. In contrast, Lo teaches that *N. gonorrhoeae* and *N. meningitidis* have high sequence homology. Thus, a person skilled in the art would expect that Lo's second sample, i.e., test dots of *N. meningitidis*, might comprise polynucleotide molecules that are also contained in the first sample. In any event, Lo clearly does not teach or suggest that its test dots of *N. meningitidis* do not contain any of the molecules present in the test dots of *N. gonorrhoeae*.

As discussed above, both the Lockhart Article and the Lockhart Patent are concerned with finding a pair of PM and MM probes that offers the optimal signal difference for determining the amount of hybridization of the target in a sample, and are not

concerned with comparing directly a probe's level of hybridization to a first sample with the probe's level of hybridization to a second sample, much less determining a ratio of the levels of hybridization and using such a ratio as a measure of the binding property of the probe. Thus, irrespective of whether the Lockhart Article or the Lockhart Patent teaches differential labeling with fluorescence labels or attaching probes to microarrays, they do not provide motivation to a person skilled in the art to modify the method of Lo in such a way as to render the presently claimed invention of claim 85 obvious.

Thus, Appellant respectfully submits that Lo, the Lockhart Article, and the Lockhart Patent, do not render claims 61-63, 66, 74-75 and 84-85 obvious. The rejection is in error; and should be reversed.

## F. CONCLUSION

For all of the reasons set forth above, Appellant respectfully requests that all of the rejections of the claims on appeal be reversed.

Respectfully submitted,

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## VIII. CLAIMS APPENDIX
## CLAIMS UNDER APPEAL
## U.S. APPLICATION NO. 09/616,849
## ATTORNEY DOCKET NO. 9301-044

27. A method for evaluating a binding property of a polynucleotide probe to a target nucleotide sequence, said polynucleotide probe comprising a predetermined nucleotide base sequence that is complementary to at least a hybridizable portion of said target nucleotide sequence, said method comprising determining a ratio of the amount of hybridization of polynucleotides in a first sample to the polynucleotide probe and the amount of hybridization of polynucleotides in a second sample to the polynucleotide probe, wherein:

(a) the first sample comprises a plurality of polynucleotide molecules comprising said target nucleotide sequence; and

(b) the second sample comprises a plurality of different polynucleotide molecules wherein each different polynucleotide molecule comprises a sequence that is different from the nucleotide sequences of any other polynucleotide molecules in said plurality of different polynucleotide molecules,

wherein at least 75% of the polynucleotide molecules in said first sample are polynucleotide molecules comprising said target nucleotide sequence, and wherein said ratio is used as a measure of said binding property, thereby evaluating said binding property of said polynucleotide probe.

29. The method of claim 27 wherein the target polynucleotide sequence in the first sample is a nucleotide sequence from a gene or gene transcript of a cell or organism, or of an mRNA, cDNA or cRNA derived therefrom.

30. The method of claim 27 wherein the plurality of different polynucleotide molecules in the second sample comprise nucleotide sequences from a plurality of genes or gene transcripts of a cell or organism.

33. The method of claim 27 wherein at least 90% of the polynucleotide molecules in said first sample are said polynucleotide molecules comprising said target nucleotide sequence.

34. The method of claim 33 wherein at least 95% of the polynucleotide molecules in said first sample are said polynucleotide molecules comprising said target nucleotide sequence.

35. The method of claim 34 wherein at least 99% of the polynucleotide molecules in said first sample are said polynucleotide molecules comprising said target nucleotide sequence.

36. The method of claim 27 wherein each different polynucleotide molecule in the second sample does not comprise the target nucleotide sequence.

37. The method of claim 36 wherein:

(a) the target polynucleotide sequence in the first sample is a sequence from a gene or gene transcript of a cell or organism; and

(b) the second sample comprises a polynucleotide sample from a deletion mutant of the cell or organism,

wherein the deletion mutant of the cell or organism does not express the gene or gene transcript.

38. The method of claim 27 wherein the plurality of different polynucleotide molecules in the second sample comprises:

(a) polynucleotide molecules comprising the target nucleotide sequence, and

(b) a plurality of different polynucleotide molecules, each comprising a different nucleotide sequence and each not comprising the target nucleotide sequence.

39. The method of claim 38 wherein:

(a) the target nucleotide sequence comprises a sequence from a gene or gene transcript of a cell or organism; and

(b) the second sample comprises a polynucleotide sample from a wild-type strain of the cell or organism,

wherein the wild-type strain of the cell or organism expresses the gene or gene transcript.

40. The method of claim 27 wherein:

(a) the first sample further comprises polynucleotide molecules that do not comprise the target nucleotide sequence; and

(b) the second sample lacks said polynucleotide molecules comprising said target nucleotide sequence.

42. The method of claim 40 wherein:

(a) the target nucleotide sequence is a sequence from a gene or gene transcript of a cell or organism;

(b) the first sample comprises a polynucleotide sample from a wild-type strain of the cell or organism which expresses the gene or gene transcript; and

(c) the second sample comprises a polynucleotide sample from a deletion mutant of the cell or organism which does not express the gene or gene transcript.

43. The method of claim 27 wherein

(a) the first sample further comprises polynucleotide molecules that do not comprise the target nucleotide sequence; and

(b) the second sample comprises:

(i) polynucleotide molecules comprising the target nucleotide sequence, and

(ii) a plurality of different polynucleotide molecules, each different polynucleotide molecule comprising a different nucleotide sequence and not comprising the target nucleotide sequence,

wherein the amount of polynucleotide molecules in the first sample comprising the target nucleotide sequence differs by at least a factor of two from the amount of polynucleotide molecules in the second sample comprising the target nucleotide sequence.

44. The method of claim 43 wherein the amount of polynucleotide molecules in the first sample comprising the target nucleotide sequence differs from the amount of polynucleotide molecules in the second sample comprising the target nucleotide sequence by at least a factor of four.

45. The method of claim 43 wherein the amount of polynucleotide molecules in the first sample comprising the target nucleotide sequence differs from the amount of polynucleotide molecules in the second sample comprising the target nucleotide sequence by at least a factor of eight.

46. The method of claim 43 wherein the amount of polynucleotide molecules in the first sample comprising the target nucleotide sequence differs from the amount of polynucleotide molecules in the second sample comprising the target nucleotide sequence by at least a factor of twenty.

47. The method of claim 43 wherein the amount of polynucleotide molecules in the first sample comprising the target nucleotide sequence differs from the amount of polynucleotide molecules in the second sample comprising the target nucleotide sequence by at least a factor of 100.

48. The method of claim 43 wherein each said polynucleotide molecule that does not comprise the target nucleotide sequence in the first sample is present in the second sample in an amount that differs from the amount of said polynucleotide molecule in the first sample by no more than a factor of 100.

49. The method of claim 43 wherein each said polynucleotide molecule that does not comprise the target nucleotide sequence in the first sample is present in the second sample in an amount that differs from the amount of said polynucleotide molecule in the first sample by no more than a factor of 10.

50. The method of claim 43 wherein each said polynucleotide molecule that does not comprise the target nucleotide sequence in the first sample is present in the second

sample in an amount that differs from the amount of said polynucleotide molecule in the first sample by no more than 50%.

51. The method of claim 43 wherein the mean abundance of the polynucleotide molecules that do not comprise the target nucleotide sequence in the first sample differs from the mean abundance of the different polynucleotide molecules that do not comprise the target nucleotide sequence in the plurality of different polynucleotide molecules of the second sample by no more than a factor of two.

52. The method of claim 43 wherein the mean abundance of the polynucleotide molecules that do not comprise the target nucleotide sequence in the first sample differs from the mean abundance of the different polynucleotide molecules that do not comprise the target nucleotide sequence in the plurality of different polynucleotide molecules of the second sample by no more than 50%.

53. The method of claim 43 wherein the mean abundance of the polynucleotide molecules that do not comprise the target nucleotide sequence in the first sample differs from the mean abundance of the different polynucleotide molecules that do not comprise the target nucleotide sequence in the plurality of different polynucleotide molecules of the second sample by no more than 10%.

54. The method of claim 43 wherein the mean abundance of the polynucleotide molecules that do not comprise the target nucleotide sequence in the first sample differs from the mean abundance of the different polynucleotide molecules that do not comprise the target nucleotide sequence in the plurality of different polynucleotide molecules of the second sample by no more than 1%.

59. The method of claim 27 wherein the polynucleotide molecules in the first sample are detectably labeled.

60. The method of claim 27 wherein the polynucleotide molecules in the second sample are detectably labeled.

61. The method of claim 59 or 60 wherein the polynucleotide molecules are labeled with a fluorescent molecule.

62. The method of claim 27 wherein:

(a) the polynucleotide molecules in the first sample are labeled with a first label; and

(b) the polynucleotide molecules in the second sample are labeled with a second label,

the first label being distinguishable from the second label.

63. The method of claim 62 wherein:

the first label is a first fluorescent molecule, and

the second label is a second fluorescent molecule.

64. The method of claim 27 wherein the polynucleotide probe is attached to a surface of a support.

65. The method of claim 27 wherein the polynucleotide probe is one of a plurality of polynucleotide probes.

66. The method of claim 65 wherein the plurality of polynucleotide probes comprises polynucleotide probes in an array of polynucleotide probes,

said array having a support with at least one surface and different polynucleotide probes attached to said surface,

wherein each of said different polynucleotide probes attached to said surface is attached to the surface of the support in a different location.

67. A method for evaluating a binding property of a plurality of polynucleotide probes to a target nucleotide sequence, wherein each polynucleotide probe in the plurality of polynucleotide probes comprises a different predetermined nucleotide base sequence that is complementary to at least a hybridizable portion of said target nucleotide sequence, said method comprising determining for each said polynucleotide probe a ratio of the amount of hybridization of polynucleotides in a first sample to said polynucleotide probe and the amount of hybridization of polynucleotides in a second sample to said polynucleotide probe, wherein:

(a) the first sample comprises a plurality of polynucleotide molecules comprising said target nucleotide sequence; and

(b) the second sample comprises a plurality of different polynucleotide molecules wherein each different polynucleotide molecule comprises a nucleotide sequence that is different from nucleotide sequence of any other polynucleotide molecules in said plurality of different polynucleotide molecules,

wherein at least 75% of the polynucleotide molecules in said first sample are polynucleotide molecules comprising said target nucleotide sequence, and wherein said ratio is used as a measure of said binding property, thereby evaluating said binding property of each said polynucleotide probe.

73. The method of claim 67 wherein each polynucleotide probe in the plurality of polynucleotide probes is attached to a surface of a support.

74. The method of claim 67 wherein the plurality of polynucleotide probes comprises polynucleotide probes in an array of probes,

said array having a support with at least one surface and different polynucleotide probes attached to said surface,

wherein each of said different polynucleotide probes attached to said surface in the plurality of probes is attached to the surface of the support in a different location.

75. The method of claim 67 wherein the first sample comprises two or more different polynucleotide molecules

wherein none of the two or more different polynucleotide molecules hybridizes or cross-hybridizes to a probe that also hybridizes or cross-hybridizes to another one of the two or more different polynucleotide molecules.

84. The method of claim 27 wherein:

polynucleotides in the first sample are labeled with a first label and polynucleotides in the second sample are labeled with a second label that is distinguishable from the first label;

and further comprising, prior to said step of comparing the steps of:

(i) concurrently contacting the polynucleotide probe with the first sample and the second sample under conditions conducive to hybridization, and

(ii) detecting any binding that occurs between the polynucleotide probe and polynucleotides in the first sample and the second sample.

85. The method of claim 84 wherein the second sample lacks polynucleotide molecules of said first sample.

90. The method of any one of claims 27, 29-30, 33-40, 42-54, 61-67, 73-75 and 84-85, wherein said polynucleotide molecules comprising said target nucleotide sequence are the same polynucleotide molecule.

91. A method for evaluating a binding property of a plurality of polynucleotide probes to a target nucleotide sequence, said method comprising determining for each said polynucleotide probe a ratio of the amount of hybridization of polynucleotides in a first sample to said polynucleotide probe and the amount of hybridization of polynucleotides in a second sample to said polynucleotide probe, wherein:

(a) said first sample comprises a plurality of polynucleotide molecules comprising said target nucleotide sequence and a plurality of polynucleotide molecules that do not comprise the target nucleotide sequence; and

(b) said second sample comprises a plurality of different polynucleotide molecules wherein each different polynucleotide molecule comprises a sequence that is different from the nucleotide sequence of any other

polynucleotide molecule in said plurality of different polynucleotide molecules, and wherein each different polynucleotide molecule in the second sample does not comprise the target nucleotide sequence,

wherein each polynucleotide probe in the plurality of polynucleotide probes comprises a different predetermined nucleotide base sequence that is complementary to at least a hybridizable portion of said target nucleotide sequence and wherein said ratio is used as a measure of said binding property, thereby evaluating said binding property of said plurality of polynucleotide probes.

92. The method of claim 91 wherein:

(a) said target nucleotide sequence is a sequence from a gene or gene transcript of a cell or organism;

(b) said first sample comprises a polynucleotide sample from a wild-type strain of the cell or organism which expresses the gene or gene transcript; and

(c) said second sample comprises a polynucleotide sample from a deletion mutant of the cell or organism that does not express the gene or gene transcript.

93. A method for evaluating a binding property of a plurality of polynucleotide probes to a target nucleotide sequence, said method comprising determining for each of said polynucleotide probes a ratio of the amount of hybridization of polynucleotides in a first sample to said polynucleotide probe and the amount of hybridization of polynucleotides in a second sample to said polynucleotide probe, wherein:

(a) said first sample comprises a plurality of polynucleotide molecules comprising said target nucleotide sequence and a plurality of polynucleotide molecules that do not comprise the target nucleotide sequence; and

(b) said second sample comprises a plurality of different polynucleotide molecules wherein each different polynucleotide molecule comprises a sequence that is different from the nucleotide sequence of any other polynucleotide molecule in said plurality of different polynucleotide molecules,

wherein each polynucleotide probe in the plurality of polynucleotide probes comprises a different predetermined nucleotide base sequence that is complementary to at least a hybridizable portion of said target nucleotide sequence and wherein said ratio is used as a measure of said binding property, thereby comparing said binding property of said plurality of polynucleotide probes.

94. The method of claim 93 wherein:

(a) said target nucleotide sequence comprises a sequence from a gene or gene transcript of a cell or organism; and

(b) said second sample comprises a polynucleotide sample from a wild-type strain of said cell or organism, wherein the wild-type strain of the cell or organism expresses the gene or gene transcript.

95. The method of claim 93, wherein said second sample comprises:

(b1) polynucleotide molecules comprising the target nucleotide sequence, and

(b2) a plurality of different polynucleotide molecules, each different polynucleotide molecule comprising a different nucleotide sequence and not comprising the target nucleotide sequence,

and wherein the amount of polynucleotide molecules in said first sample comprising the target nucleotide sequence differs by at least a factor of two from the amount of polynucleotide molecules in said second sample comprising the target nucleotide sequence.

96. The method of claim 95 wherein the amount of polynucleotide molecules in said first sample comprising said target nucleotide sequence differs from the amount of polynucleotide molecules in said second sample comprising said target nucleotide sequence by at least a factor of four.

97. The method of claim 95 wherein the amount of polynucleotide molecules in said first sample comprising said target nucleotide sequence differs from the amount of polynucleotide molecules in said second sample comprising said target nucleotide sequence by at least a factor of eight.

98. The method of claim 95 wherein the amount of polynucleotide molecules in said first sample comprising said target nucleotide sequence differs from the amount of polynucleotide molecules in said second sample comprising said target nucleotide sequence by at least a factor of twenty.

99. The method of claim 95 wherein the amount of polynucleotide molecules in said first sample comprising said target nucleotide sequence differs from the amount of

polynucleotide molecules in said second sample comprising said target nucleotide sequence by at least a factor of 100.

100. The method of claim 95 wherein each said polynucleotide molecule that does not comprise said target nucleotide sequence in said first sample is present in said second sample in an amount that differs from the amount of said polynucleotide molecule in the first sample by no more than 50%.

101. The method of claim 95 wherein the mean abundance of the polynucleotide molecules that do not comprise the target nucleotide sequence in the first sample differs from the mean abundance of the different polynucleotide molecules that do not comprise the target nucleotide sequence in the plurality of different polynucleotide molecules of the second sample by no more than a factor of two.

102. The method of claim 95 wherein the mean abundance of the polynucleotide molecules that do not comprise the target nucleotide sequence in the first sample differs from the mean abundance of the different polynucleotide molecules that do not comprise the target nucleotide sequence in the plurality of different polynucleotide molecules of the second sample by no more than 50%.

103. The method of claim 95 wherein the mean abundance of the polynucleotide molecules that do not comprise the target nucleotide sequence in the first sample differs from the mean abundance of the different polynucleotide molecules that do not comprise the target nucleotide sequence in the plurality of different polynucleotide molecules of the second sample by no more than 10%.

104. The method of claim 95 wherein the mean abundance of the polynucleotide molecules that do not comprise the target nucleotide sequence in the first sample differs from the mean abundance of the different polynucleotide molecules that do not comprise the target nucleotide sequence in the plurality of different polynucleotide molecules of the second sample by no more than 1%.

## IX. EVIDENCE APPENDIX

None.

## XI. RELATED PROCEEDINGS APPENDIX

None.